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Lamotrigine blocks NMDA receptor-initiated arachidonic acid signalling in rat brain: Implications for its efficacy in bipolar disorder

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Abstract

An upregulated brain arachidonic acid (AA) cascade and a hyperglutamatergic state characterize bipolar disorder (BD). Lamotrigine (LTG), a mood stabilizer approved for treating BD, is reported to interfere with glutamatergic neurotransmission involving N-methyl-D-aspartate receptors (NMDARs). NMDARs allow extracellular calcium into the cell, thereby stimulating calcium-dependent cytosolic phospholipase A_2 (cPLA_2) to release arachidonic acid (AA) from membrane phospholipid. We hypothesized that LTG, like other approved mood stabilizers, would reduce NMDAR-mediated AA signaling in rat brain. An acute subconvulsant dose of NMDA (25 mg/kg) or saline was administered intraperitoneally to unanesthetized rats that had been treated p.o. daily for 42 days with vehicle or a therapeutically relevant dose of LTG (10 mg/kg/.d). Regional brain AA incorporation coefficients k* and rates J_{in}, AA signals, were measured using quantitative autoradiography after intravenous [1-^{14}C]AA infusion, as were other AA cascade markers. In chronic vehicle-treated rats, acute NMDA compared to saline increased k* and J_{in} in widespread regions of the brain, as well as prostaglandin (PG)E_2 and thromboxane B_2 concentrations. Chronic LTG treatment compared to vehicle reduced brain cyclooxygenase (COX) activity, PGE_2 concentration, and DNA binding activity of the COX-2 transcription factor, NF-κB. Pretreatment with chronic LTG blocked the acute NMDA effects on AA cascade markers. In summary, chronic LTG like other mood stabilizers blocks NMDA-mediated signaling involving the AA metabolic cascade. Since markers of the AA cascade and of NMDAR signaling are up-regulated in the postmortem BD brain, mood stabilizers generally may be effective in BD by dampening NMDAR signalling and the AA cascade.

Keywords

arachidonic acid; cyclooxygenase; mood stabilizer; NMDA receptor; phospholipase A_2

INTRODUCTION

Hyperglutamatergic neurotransmission has been implicated in bipolar disorder (BD) (Cherlyn *et al.*, 2010; Clinton and Meador-Woodruff, 2004; Michael *et al.*, 2003; Zarate *et al.*, 2003). Furthermore, the postmortem BD brain shows fewer glutamatergic N-methyl-D-

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Statement of Interest
None.

aspartate receptors (NMDARs), decreased protein and mRNA levels of NMDAR subunits NR1 and NR3A, reduced densities of NMDAR-associated postsynaptic proteins, PSD-95 and SAP102, and increased expression of the vesicular glutamate transporter 1 (Eastwood and Harrison, 2010; Hashimoto *et al.*, 2007; McCullumsmith *et al.*, 2007; Mueller and Meador-Woodruff, 2004; Rao *et al.*, 2010). It has been shown that NMDAR stimulation by glutamate or NMDA decreases NR-1 expression (Gascon *et al.*, 2005), and that the NR3A subunit co-assembles with other subunits (NR1, NR2A or NR2B) to form NMDARs with decreased activity and calcium influx (Ciabarra *et al.*, 1995; Sucher *et al.*, 1995). Further, chronic sub-convulsive administration of NMDA to rats decreased NR-1 and NR3A NMDA receptor subunits along with upregulated arachidonic acid (AA) selective cytosloic phospholipase A_2 activity, protein and mRNA levels in rat frontal cortex (Rao *et al.*, 2007a). Decreased NR1 and NR3A expression observed in BD is consistent with up-regulated NMDAR function (Kim et al., 2011; Rao et al., 2010), and with the observation that mice lacking the NR-3A subunit have increased brain NMDAR activity (Das *et al.*, 1998).

When glutamate or NMDA binds to an NMDAR, extracellular Ca^{2+} enters the cell and activates, among other enzymes, Ca^{2+}-dependent-cytosolic phospholipase A_2 type IV (cPLA$_2$-IV), which selectively releases arachidonic acid (AA, 20:4n-6) from cell membrane phospholipids (Basselin *et al.*, 2006a; Basselin *et al.*, 2008; Basselin *et al.*, 2007a; Clark *et al.*, 1991; Dumuis *et al.*, 1988; Ramadan *et al.*, 2010). Consistent with a hyperglutamatergic state, the postmortem BD brain shows up-regulated markers of AA metabolism, including cPLA$_2$, cyclooxygenase (COX)-2, and membrane prostaglandin E synthase, which converts AA to pro-inflammatory prostaglandin (PG)E_2 (Kim *et al.*, 2011).

The brain AA signal involving NMDARs has been imaged in unanesthetized rats by infusing [1-^{14}C]AA intravenously and measuring regional brain radioactivity following injection of NMDA (Basselin *et al.*, 2007c). Operational equations, derived with a fatty acid model (Rapoport, 2001; Robinson *et al.*, 1992), were used to identify the signal as increased regional AA incorporation coefficients k* or fluxes J_{in}. Acute administration of a subconvulsive dose of NMDA (25 mg/kg, i.p.) increased k* and J_{in} for AA in many brain regions. The increases could be blocked by pretreatment with the specific NMDAR antagonist, MK-801, or with each of three mood stabilizers effective against BD, namely lithium, valproate or carbamazepine (Basselin *et al.*, 2006a; Basselin *et al.*, 2008; Basselin *et al.*, 2007a). We have also found that lithium and carbamazepine, when administered chronically at therapeutically relevant concentrations, reduced mRNA, protein, and activity levels of cPLA$_2$-IV, and each of the three drugs, as well as lamotrigine (LTG), another FDA-approved mood stabilizer (Bowden, 2005; FDA, 2009), decreased protein and mRNA of COX-2 in rat brain (Lee *et al.*, 2008; Rao *et al.*, 2007b; Rao *et al.*, 2007c; Rao *et al.*, 2005; Rapoport *et al.*, 2009).

LTG [3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine] is thought to act on voltage-dependent Na^+ and Ca^{2+} channels so as to reduce presynaptic neuronal depolarization, and thus glutamate release at the excitatory synapse (Cunningham and Jones, 2000; Sitges *et al.*, 2007a; Sitges *et al.*, 2007b; Xie and Hagan, 1998). Further, LTG can modulate neurotransmission *via* NMDARs (Anand *et al.*, 2000; Farber *et al.*, 2002; Wang *et al.*, 1996).

In the present study, we hypothesized that LTG, like lithium, valproate and carbamazepine, when given chronically to rats (Basselin *et al.*, 2006a; Basselin *et al.*, 2008; Basselin *et al.*, 2007a), would block NMDAR-initiated AA signaling in rat brain, and dampen other parameters of the AA metabolic cascade stimulated by NMDA. Confirming this would support our hypothesis that a common mechanism for the action of FDA-approved mood stabilizers in BD is down-regulation of the brain AA cascade that involves inhibition of NMDAR-mediated AA signaling (Rapoport *et al.*, 2009; Rapoport and Bosetti, 2002). To

test this hypothesis, we used our fatty acid method to measure incorporation coefficients k* and rates J_{in} for AA in 83 brain regions of unanesthetized rats that were injected acutely with saline or NMDA, and which had received LTG or vehicle daily for 42 days as described previously (Lee *et al.*, 2008). Brain $cPLA_2$-IV and COX activities, nuclear factor (NF)-κB DNA binding activity, and PGE_2 and thromboxane $(TX)B_2$ concentrations, also were measured.

MATERIALS AND METHODS

Animals and Diets

Male Fischer-344 rats, aged 2 months (Taconic Farms, USA) were acclimated for 1 wk in an animal facility with regulated temperature, humidity and light cycle, and with free access to food and water. They were fed Rodent NIH-31 Auto 18-4 diet (Zeigler Bros, Gardens, PA), which contained (as % total fatty acid), 20.1% saturated, 22.5% monounsaturated, 47.9% linoleic, 5.1% α-linolenic, 0.02% arachidonic, 2.0% eicosapentaenoic, and 2.3% docosahexaenoic acid (Demar *et al.*, 2005). Experiments followed the "Guide for the Care and Use of Laboratory Animals" (National Institutes of Health, Publication No. 86–23), and were approved by the Animal Care and Use Committee of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development.

Drugs and Tracers

Radiolabeled [1-^{14}C]AA in ethanol (53 mCi/mmol, 99.4% pure, Moravek Biochemicals, USA) was evaporated and resuspended in Hepes buffer (pH 7.4), containing 50 mg/ml fatty acid-free bovine serum albumin (Sigma-Aldrich, USA) (DeGeorge *et al.*, 1989). LTG (provided by NIMH Chemical Synthesis and Drug Supply Program, RTI International, USA)-treated rats received 10 mg/kg LTG by gavage once daily for 42 days. LTG was dissolved in a 1:1 volume dimethylsulfoxide (DMSO, ≥ 99% Sigma-Aldrich)/saline (0.9% sodium chloride) solution. This regimen produced a serum LTG concentration of 40 μM (Hassel *et al.*, 2001), at the high end of concentrations reported in humans (20–30 μM) (Doose *et al.*, 2003). Vehicle-treated rats (controls) received an equivalent volume of DMSO/saline.

Surgical Procedures and Tracer Infusion

On the morning of day 42, a rat was gavaged with its appropriate treatment at 1 h before undergoing anesthesia with halothane (2–3% v/v in O_2). Polyethylene PE-50 catheters were surgically implanted into its right femoral artery and vein (Basselin *et al.*, 2006a), and the rat was allowed to recover from anesthesia (3–4 h) in a temperature-controlled and sound-dampened box, while body temperature was maintained at 36.4 – 37.1°C using a feedback heating device and rectal thermometer. Arterial blood pressure and heart rate were measured with a blood pressure recorder. Ten minutes after injecting NMDA or saline, [1-^{14}C]AA (2 ml, 170 μCi/kg) was infused into the femoral vein for 5 min with an infusion pump, at a rate of 400 μl/min. Twenty minutes after starting infusion, the rat was killed with an overdose of Nembutal (90 mg/kg, i.v.) and decapitated. The brain was removed quickly, frozen in 2-methylbutane maintained at −40°C on dry ice, and stored at −80°C for later sectioning.

Chemical Analysis

Blood samples, collected before, during or after [1-^{14}C]AA infusion, were centrifuged at 18 000 *g* for 30 s. Total lipids were extracted from plasma (30 μl) using a modified Folch procedure (Folch *et al.*, 1957). One hundred microlitres of the lower organic phase of the solution was used to determine the radiolabelled unesterified plasma AA concentration by

liquid scintillation counting. As reported previously (DeGeorge *et al.*, 1989), > 95–98% of plasma radioactivity following [1-^{14}C] AA infusion was radiolabelled AA.

Unlabeled, unesterified fatty-acid concentrations also were determined in frozen arterial plasma (100 μl). Total lipids were extracted (Folch *et al.*, 1957) and separated by thin-layer chromatography on 60 silica gel plates with heptane:diethylether:glacial acetic acid 60:40:3 (v/v/v). Unesterified fatty acids were scraped from the plate and converted to methyl ester derivatives (1% H_2SO_4 in methanol, 3 h, 70°C), which then were analyzed by gas chromatography with flame ionization detection, and quantified relative to the added standard, heptadecanoic acid (17:0).

Quantitative Autoradiography

Quantitative autoradiography was performed as reported previously (Basselin *et al.*, 2006a). Radioactivity (nCi/g wet brain) in 83 bilateral anatomically identified regions (Paxinos and Watson, 1987) was determined by quantitative densitometry (NIH Image 1.62). Regional brain AA incorporation coefficients k* (ml plasma/s/g brain) were calculated as (Robinson *et al.*, 1992),

$$k^* = \frac{c^*_{brain}(20\ \text{min})}{\int_o^T c^*_{pl}\, dt} \quad (1)$$

where c^*_{brain} (nCi/g wet brain wt) is brain radioactivity 20 min after beginning infusion, c^*_{plasma} (nCi/ml plasma) is labelled plasma unesterified AA, and t (min) is time after the beginning of [1-^{14}C] AA infusion. Plasma radioactivity was integrated by trapezoidal integration to calculate k* for each experiment. Regional rates of incorporation of unesterified AA from plasma into brain phospholipids, J_{in} (nmol/s/g) were calculated as,

$$J_{\text{in}} = k^* c_{plasma} \quad (2)$$

where c_{plasma} is the plasma concentration (nmol/ml) of unlabeled unesterified AA.

Activity of cPLA$_2$ Type IV

In separate experiments, after the last LTG or vehicle dose was administered, a rat was anesthetized with Nembutal (50 mg/kg i.p.) and decapitated. The brain was removed, frozen in 2-methylbutane maintained at −40°C on dry ice, and stored at −80°C. Half of the brain was homogenized at 4°C using a Tenbroeck tissue grinder, in 2 vol cold buffer containing 10 mM Hepes (pH7.5), 1 mM EDTA, 0.34 M sucrose and protease inhibitor cocktail tablet (Complete, Germany). Homogenates were centrifuged (14,000 *g* for 20 min, then 100,000 *g* for1 h, 4°C), and supernatants corresponding to the cytosolic fractions were assayed for cPLA$_2$-IV activity (Yang *et al.*, 1999). Protein concentrations in the cytosolic fraction were determined by the Bradford method (Bradford, 1976).

COX Activity

Half of each brain was homogenized using a Tenbroeck tissue grinder in 3 ml of ice-cold lysate buffer at pH 7.8, containing 10 mM Tris-HCl, 0.15 M NaCl, 1 mM EDTA and 1% Igepal CA-630, and the homogenates centrifuged (14,000 *g*, 20 min, 4°C). Net COX activity was measured as the rate of PGE_2 formation (pg PGE_2/min.mg cytosolic protein) in the homogenate cytosolic fractions, as reported previously (Basselin *et al.*, 2009). As a positive control, the specific COX-2 inhibitor Celebrex® (400 mg; Pfizer Inc., USA, Division of Veterinary Medicine, USA) was dissolved in DMSO at a concentration of 0.1% (w/v), and added to the mixture 10 min before adding AA (Basselin *et al.*, 2009).

Nuclear Extracts and NF-κB DNA Binding Activity

Nuclear extracts from the frontal cortex of either the chronic vehicle- or LTG-treated animals, prepared as reported (Lahiri, 1998; Rao *et al.*, 2007b), were analyzed by gel shift assays to quantify DNA binding activity of NF-κB (Kaltschmidt *et al.*, 2002).

Eicosanoid Concentrations

Rat brains were prepared, microwaved and used as reported previously (Basselin *et al.*, 2008; Basselin *et al.*, 2007a). Eicosanoid concentrations were measured with polyclonal PGE_2 and TXB_2 ELISA assay kits (Oxford Biochemical Research, USA).

Statistical Analysis

A paired *t* test using GraphPad Prism version 4.0b (GraphPad Software, USA) was used to compare mean physiological parameters in the same animal before and after drug injection, and an unpaired two-tailed *t*-test was used to compare mean physiological parameters in LTG- and vehicle-treated rats. A standard two-way analysis of variance (ANOVA) was performed to compare chronic LTG and vehicle treatment with acute NMDA vs. saline administration, with regard to integrated arterial plasma radioactivity, unesterified plasma fatty-acid concentrations, brain PGE_2 and TXB_2 concentrations and regional values of k* and J_{in} for AA. If interactions between LTG and NMDA were statistically insignificant, probabilities of main effects of LTG and NMDA are reported. If the interactions were statistically significant, these probabilities are not reported (Tabachnick and Fidell, 2001). A one-way ANOVA with Bonferroni's *post-hoc* test also was used to compare NMDA and saline responses between chronic LTG- and vehicle-treated rats, as well as saline responses in LTG-treated compared with vehicle-treated rats. Data are reported as mean ± SD, with statistical significance taken at $p \leq 0.05$.

RESULTS

Physiology and Arterial Plasma Radioactivity

Mean body weights of 42-d LTG-treated rats did not differ significantly from weight of the vehicle-treated rats [267.5 ±18.1 g (n = 16) vs. 265.5 ±18.0 g (n = 16)], as reported (Daoud *et al.*, 2004; Hassel *et al.*, 2001). There also was no significant group difference in mean rectal temperature, heart rate or arterial blood pressure (data not shown). Acute NMDA decreased heart rate by 21–23% ($p < 0.0001$) in the chronic LTG- and vehicle-treated rats (data not shown), as reported by Basselin et al. (2006a, 2007a, 2008).

Neither chronic LTG nor acute NMDA modified the time course of arterial plasma radioactivity [equation (1)] during i.v. [1-^{14}C]AA infusion. Integrated radioactivity in the plasma organic fraction (nCi × s)/(ml plasma) (n = 7–9), the input function in equation (1), did not differ significantly among groups: chronic vehicle + saline, 147,543 ± 30,055; chronic vehicle + NMDA, 150,018 ± 22,506; chronic LTG + saline, 149,499 ± 43,167; chronic LTG + NMDA, 145,409 ± 32,774.

Plasma Concentrations of Unlabeled Unesterified Fatty Acid

A two-way ANOVA showed a significant main effect of LTG on plasma concentrations of unesterified palmitic, palmitoleic, stearic, oleic, linoleic, α-linolenic, arachidonic and docosahexaenoic acid, which were reduced in chronic LTG-treated rats compared to the chronic vehicle-treated rats ($p<0.05$). No significant main effect of NMDA, or interaction between LTG and NMDA, was detected (Table 1).

Regional Brain AA Incorporation Coefficients, k*

Figure 1 presents representative coronal autoradiographs of brains from rats treated for 42 d with vehicle (control) or with LTG, then acutely injected with saline or NMDA. k* for AA, calculated by equation (1), is colour-coded. The figure shows no evident difference in regional values of k* in response to acute saline between animals treated chronically with LTG compared with vehicle. Acute NMDA evidently increased k* in multiple brain regions of the chronic saline-injected but not LTG-injected rats. Data such as illustrated in Fig. 1 were collated in Table 2.

Mean AA incorporation coefficients k* in each of 83 brain regions were subjected to a two-way ANOVA (Table 2). Statistically significant interactions between LTG and NMDA were found in 52 regions, for each of which a one-way ANOVA with Bonferroni's *post-hoc* test showed that chronic LTG did not significantly change mean baseline (after saline) k* (Table 2). In 37 of the 52 regions, the same one-way ANOVA showed that NMDA compared with saline significantly increased k* by 29–59% in chronic vehicle-treated rats. Affected regions included pyriform (55%), motor (49–53%), sensory cortical areas (39–46%), preoptic area (58%), diagonal band (48%), amygdala (52%), hippocampus [CA1, CA2, CA3, dentate gyrus] (31–45%), nucleus accumbens (42%), caudate-putamen (39–46%), lateral habenular nucleus (41%), thalamus [ventroposterior lateral, ventroposterior medial, anteroventral and parafascicular nuclei] (46–50%), hypothalamus [supraoptic, periventricular, arcuate, ventromedial, posterior nuclei] (35–51%), zona incerta (47%), and the subfornical organ (58%). However, NMDA compared to saline did not significantly increase k* in any of the 37 regions in chronic LTG-treated rats.

In none of the 31 regions having a statistically insignificant LTG × NMDA interaction did LTG or NMDA have a significant main effect on k* for AA (data not shown). Thus, chronic LTG prevented each of the 37 significant NMDA-induced k* increments that were observed in the chronic vehicle-treated rats, but did not have a significant effect in naive rats (Table 2 and Fig. 1).

Regional Rates of Incorporation of Unlabeled Unesterified AA into Brain

Baseline (after saline) and NMDA-induced regional values of J_{in} (products of k* and the unesterified plasma AA concentration) were calculated by equation (2). A two-way ANOVA showed statistically significant interactions between LTG and NMDA in 44 of the 83 regions that were examined (data not shown). In 32 of these, a one-way ANOVA and Bonferroni's *post-hoc* test showed that acute NMDA compared with saline significantly increased J_{in} (by 7–65%) in chronic vehicle- but not chronic LTG-treated rats. In the 39 regions where LTG × NMDA interactions with regard to J_{in} were statistically insignificant, chronic LTG compared to vehicle had a negative main effect (10 of the 39), largely reflecting the reduced plasma AA concentration following LTG.

Brain Enzyme and NF-κB Binding Activities

Since cPLA$_2$-IV selectively releases AA from membrane phospholipids, we measured the enzymatic activity of this protein. Whole brain cPLA$_2$-IV activity did not differ significantly ($p = 0.91$) between rats that received chronic LTG compared with vehicle (Fig. 2*a*). Since a Ca^{2+} chelator is used to determine cPLA$_2$ activity *in vitro* (see Methods), we did not measure brain cPLA$_2$-IV activity following acute NMDA injection, because the elevated intracellular Ca^{2+} concentration associated with NMDA administration *in vivo* could not be produced reliably by NMDA *in vitro* (Clark *et al.*, 1991).

Our previous study showed that chronic LTG down-regulated protein and mRNA of COX-2 by 50% in rat brain in the absence of a decrease of COX-1 (Lee *et al.*, 2008). We therefore

determined whether COX activity was altered after LTG treatment. As shown in Fig. 2*b*, brain COX activity was decreased significantly by 56% ($p < 0.001$) in chronic LTG-treated compared with vehicle-treated rats. Compared with vehicle, Celebrex® (100 μM) inhibited COX activity by 70% (25.3 ± 3.0 vs. 84.8 ± 10.5 pg/min.mg protein, n = 8, data not shown), as has been reported (Basselin *et al.*, 2009). To consider the transcriptional mechanism underlying the decreased COX-2 expression, we measured the DNA binding activity of NF-κB, a major transcription factor for COX-2 (Kaltschmidt *et al.*, 2002). Figure 2*c* illustrates that chronic LTG significantly decreased by 38% ($p < 0.001$) the binding of nuclear proteins to the NF-κB consensus sequence of NF-κB. In the presence of excess (100 times) unlabelled specific NF-κB oligonucleotides, binding of labelled oligonucleotides was blocked, confirming specificity of the DNA binding activity.

Brain Eicosanoid Concentrations

To examine the effect of reduced COX-2 activity on COX-derived products, concentrations of PGE_2 and TXB_2 were determined in microwaved brains. A two-way ANOVA demonstrated statistically significant interactions between chronic LTG and acute NMDA with regard to brain PGE_2 and TXB_2 concentrations (Table 3). A subsequent one-way ANOVA with Bonferroni's *post-hoc* test showed that chronic LTG reduced the basal PGE_2 concentration by 52 % ($p < 0.05$), without affecting the TXB_2 concentration. Acute NMDA significantly increased brain PGE_2 ($p < 0.001$) and TXB_2 ($p = 0.007$) concentrations by 2.2- and 2.4-fold, respectively, in chronic vehicle-treated rats, but did not affect either concentration in chronic LTG-treated rats.

DISCUSSION

Oral administration of LTG (10 mg/kg) daily for 42 d to rats, sufficient to produce a plasma LTG concentration therapeutically relevant to BD (Doose *et al.*, 2003), blocked increments in k* and J_{in} for AA and in brain PGE_2 and TXB_2 concentrations that could be produced by acute NMDA administration (25 mg/kg i.p.) in vehicle-treated rats. Chronic LTG treatment compared with vehicle did not change baseline k* or J_{in} in any of 83 brain regions examined, nor brain $cPLA_2$-IV activity or TXB_2 concentration, but did reduce brain COX activity, NF-κB DNA binding activity and PGE_2 concentration. Chronic LTG treatment also had a negative main effect on the plasma concentrations of each of eight measured unesterified fatty acids. These observations support our hypothesis that chronic LTG treatment blocks NMDA-signaling involving AA and its metabolites.

As similar effects on the NMDA-induced AA signal have been reported following administration of MK-801, a specific NMDAR antagonist, or of lithium, valproate or carbamazepine to rats (Basselin *et al.*, 2006a, 2007a, 2008; Rapoport *et al.*, 2009), these results support the hypothesis that mood stabilizers effective against BD commonly down-regulate the brain AA cascade, and provide one plausible mechanism for this effect, blockade of NMDAR-mediated activation of the cascade (Rapoport and Bosetti, 2002; Rapoport *et al.*, 2009).

Chronic LTG's suppression of the NMDA-induced increases in k* and J_{in} for AA and in PGE_2 and TXB_2 concentrations, which were produced in chronic vehicle-treated rats, could have been due to LTG's reduction of COX-2 expression. Indeed, chronic LTG decreased COX activity, DNA binding activity of NF-κB, PGE_2 and TXB_2 concentrations, consistent with a report showing down-regulation of mRNA and protein of COX-2 in rat brain (Lee *et al.*, 2008). Pharmacological inhibition or knocking out the COX-2 gene in rodent brain also reduced increments in k* for AA and in PGE_2 concentration caused by giving drugs acting at $cPLA_2$-coupled neuroreceptors (Basselin *et al.*, 2006b, 2007b). Similar to LTG, chronic administration of lithium, valproate or carbamazepine to rats decreased brain COX activity

and PGE_2 concentration (Rapoport *et al.*, 2009). When considering the possible role for inflammatory processes in BD with increased brain interleukin-1®, NF-κB subunits (p50 and p65), COX-2, membrane prostaglandin E synthase, and astroglial and microglial markers (glial fibrillary acidic protein, inducible nitric oxide synthase, and CD11b) (Kim *et al.*, 2011; Kim *et al.*, 2010; Rao *et al.*, 2010), it is important to note that the four mood stabilizers, lithium, valproate, carbamazepine, and LTG suppress brain COX-2, PGE_2, and/or NF-κB DNA binding activity which can be viewed as protecting against inflammatory processes (Lee *et al.*, 2008; Rapoport *et al.*, 2009). In contrast, topiramate, which appeared effective in Phase II trials in BD, but later failed Phase III placebo-controlled trials (Kushner *et al.*, 2006), did not change markers of the rat brain AA cascade (Ghelardoni *et al.*, 2005; Lee *et al.*, 2005). Topiramate has not been tested with regard to the NMDA signal.

Chronic LTG's effect on the NMDAR itself is unlikely to have influenced the brain AA signal in response to NMDA. Unlike lithium, carbamazepine or valproic acid (Basselin *et al.*, 2006a, 2007a, 2008), however, LTG does not affect glutamate binding to the NMDAR (Xie and Hagan, 1998), nor protein expression or activity of protein kinase C (Hahn *et al.*, 2004), which can phosphorylate the NMDAR (Leonard and Hell, 1997), suggesting that LTG employs different intracellular mechanisms (e.g. reduction in glutamate release following inhibition of Na^+ channels), in addition to the COX pathway (see above), for long-term changes in NMDAR-initiated AA signaling. The BD brain shows increased glutamate signalling associated with up-regulated markers of AA metabolism and of excitotoxicity, reduced brain-derived neurotrophic factor (BDNF), and apoptosis (Kim *et al.*, 2010, 2011; Rao *et al.*, 2010) and there is clinical evidence of hyperglutamatergic function (Cherlyn *et al.*, 2010; Clinton *et al.*, 2004; Michael *et al.*, 2003; Zarate *et al.*, 2003). Thus, down-regulation by LTG of NMDAR-initiated AA signaling and of COX activity, and of formation of pro-inflammatory PGE_2, may contribute to LTG's efficacy in BD and to its reported neuroprotective properties (see below). Recent studies indicated that LTG reduced brain glutamine levels in depressed BD patients (Frye *et al.*, 2007), and that memantine, a NMDAR antagonist, was beneficial in BD patients (Koukopoulos *et al.*, 2010; Teng and Demetrio, 2006). As a rationale for applying agents that modulate the glutamatergic system in treating patients with BD, riluzole, an inhibitor of glutamate release, has been reported to be effective alone (Brennan *et al.*, 2010) or in combination with lithium in open-label trials for the treatment of bipolar depression (Zarate *et al.*, 2005). In healthy subjects, LTG decreased perceptual abnormalities induced by ketamine, an NMDAR antagonist (Anand *et al.*, 2000).

As with lithium and valproate, exposing neuroblastoma or cerebral cortical cells to LTG inhibited glycogen synthase kinase-3β and increased activity of glutathione S-transferase, an antioxidant enzyme (Bakare *et al.*, 2009; Hayes and Strange, 2000; Li *et al.*, 2002; Strange *et al.*, 2001). LTG increased brain mRNA and protein levels of BDNF and of anti-apoptotic factor B-cell lymphoma-2 (Bcl-2), and reversed stress-induced downregulation of BDNF in rat brain (Chang *et al.*, 2009; Li *et al.*, 2010). Given that the BD brain shows reduced expression of BDNF and of other neurotrophic factors, and increased markers of apoptosis (Dwivedi *et al.*, 2003; Kim *et al.*, 2010; Knable *et al.*, 2004), these actions may contribute to neuroprotection by LTG in BD (Ketter *et al.*, 2003; Rapoport *et al.*, 2009), and in experimental models of cerebral ischemia and glutamate-induced excitotoxicity (Bacher and Zornow, 1997; Lees and Leach, 1993; Maj *et al.*, 1998; Rataud *et al.*, 1994).

The statistically insignificant effects of chronic LTG on baseline k* and J_{in} for AA and on baseline $cPLA_2$-IV activity agree with prior reports that chronic LTG did not alter rat brain $cPLA_2$-IV mRNA or protein levels (Bazinet *et al.*, 2006; Chang *et al.*, 1996; Ghelardoni *et al.*, 2004; Lee *et al.*, 2008; Lee *et al.*, 2007; Rintala *et al.*, 1999). Under basal conditions, LTG does not alter resting membrane potential, neuronal excitability, or low-frequency

excitatory synaptic transmission (Xie and Hagan, 1998), or glutamate release in the hippocampus of freely moving rats (Ahmad *et al.*, 2004). In this study, we did not determine effects of an acute LTG dose of 10 mg/kg, since this dose produces plasma LTG concentration around 11–13 μM (Ahmad *et al.*, 2004), far below the therapeutic level (Hassel *et al.*, 2001).

The baseline values of k* and J_{in} in this study agree with reported values, as do the increments in these parameters and in PGE_2 and TXB_2 concentrations following acute NMDA (Basselin *et al.*, 2007a, 2008; Okada *et al.*, 2000; Pepicelli *et al.*, 2002). Given that J_{in} represents incorporation of unesterified plasma AA as compensation for AA metabolic loss in brain (Basselin *et al.*, 2007b; Rapoport, 2003), our data indicate comparable baseline AA loss but reduced NMDA-initiated loss following chronic LTG. Lee *et al.* (2007) reported that chronic LTG did not significantly change the plasma concentration of any unlabelled unesterified fatty acids, but in our study, this effect was statistically significant ($p < 0.05$) for the eight fatty acids analyzed. This discrepancy may be due to differences in gavage, fasting time, or quality of the unlabelled fatty-acid standards. The reduced unesterified plasma fatty-acid concentrations caused by LTG may reflect a liver effect of drug, since chronic LTG also reduces plasma levels of cholesterol and triglycerides (Daoud *et al.*, 2004). Circulating unesterified fatty acids are derived by hydrolysis of esterified fatty acids that are secreted by the liver and from adipose tissue (Gao *et al.*, 2009; Gibbons *et al.*, 2004; Purdon *et al.*, 1997).

In summary, 42 d of daily LTG blocked acute NMDA-initiated brain signaling *via* AA in unanesthetized rats. This observation, taken with reports that lithium, valproate and carbamazepine have a comparable action (Basselin *et al.* 2006a, 2007a, 2008), support our hypothesis that mood stabilizers effective in BD have a common mechanism of action that involves down-regulation of the brain AA cascade and interference with NMDAR receptor signaling *via* AA (Rapoport *et al.*, 2009; Rapoport & Bosetti, 2002). Studying the AA cascade and the NMDAR signal in unanesthetized rats, as a common target for these agents, might help to screen new and potentially clinically relevant therapeutic agents for BD.

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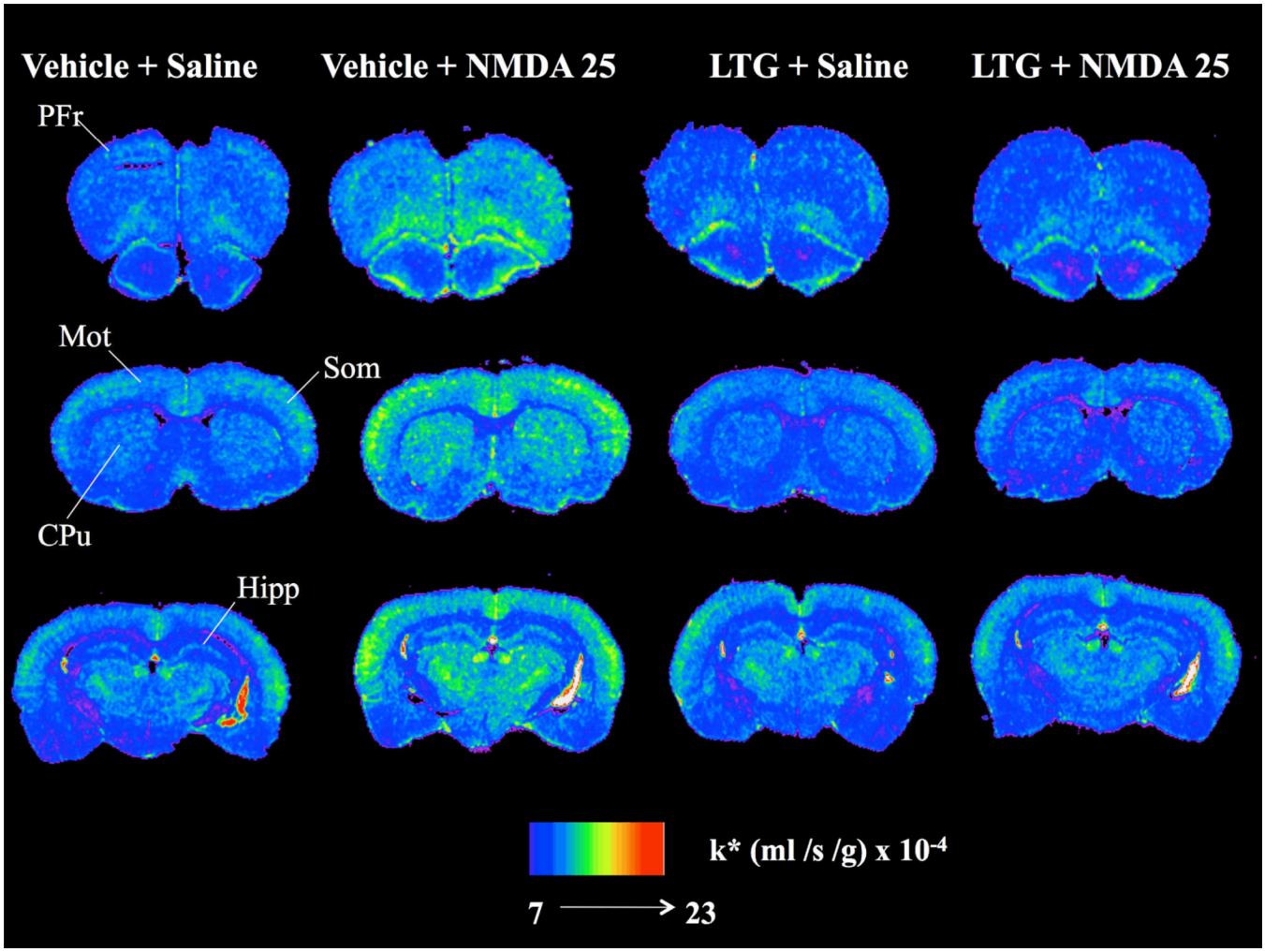
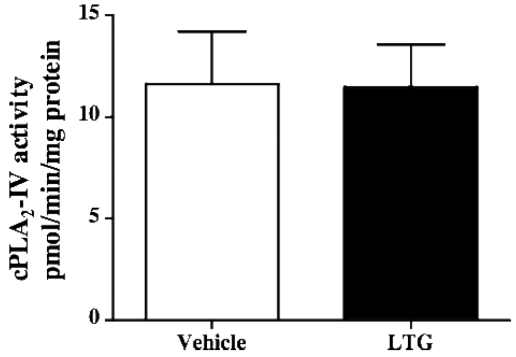


Figure 1.
Coronal brain autoradiographs showing effects of NMDA and LTG on regional AA incorporation coefficients k* in rats. Values of k* (ml/s/g brain) × 10^{-4} are given on a color scale from 7 (purple) to 23 (yellow-orange). CPu, caudate putamen; Hipp, hippocampus; LTG, lamotrigine; NMDA, N-methyl-D-aspartic acid, 25 mg/kg, i.p.; Mot, motor cortex; PFr, prefrontal cortex; Som, somatosensory cortex. NMDA, N-methyl-D-aspartate, 25 mg/kg, i.p..

(A)



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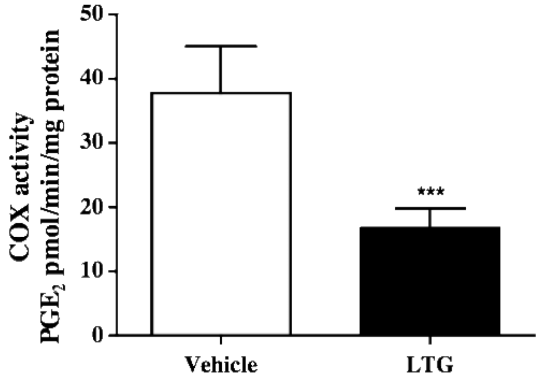

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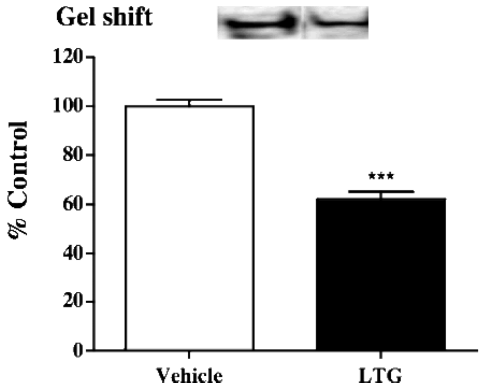

Figure 2.

Effects of chronic LTG on $cPLA_2$, COX, and NF-kB DNA binding activities.

(A) Effects of chronic LTG on whole brains: $cPLA_2$-IV activity. Data are means ± SD (n=8) and were analyzed with unpaired t-test.

(B) Effects of chronic LTG on whole brain cyclooxygenase (COX) activity. Data are means ± SD (n=8) and were analyzed with unpaired t-test. *** $p < 0.001$. PGE_2, prostaglandin E_2.

(C) Effects of chronic LTG on NF-kB DNA binding activity. Values are represented as % control (n = 8). *** $p < 0.001$.

Table 1

Effects of NMDA and Lamotrigine on Unesterified Plasma Fatty Acid Concentrations

Fatty Acid	Chronic Vehicle		Chronic LTG		LTG X NMDA Interaction (p value)	LTG effect (p value)	NMDA effect (p value)
	Saline (n=7)	NMDA (n=9)	Saline (n=8)	NMDA (n=8)			
	(nmol/ml plasma)						
Palmitic (16:0)	469.5 ± 80.1	415.5 ± 72.0	258.8 ± 64.2	326.6 ± 114.2	0.0610	< 0.0001	0.8266
Palmitoleic (16:1)	73.5 ± 19.9	61.4 ± 19.7	32.8 ± 7.0	43.9 ± 19.1	0.0710	< 0.0001	0.7796
Stearic (18:0)	76.7 ± 16.3	63.9 ± 12.1	52.6 ± 20.0	54.9 ± 14.6	0.2174	0.0099	0.3877
Oleic (18:1 n-9)	265.0 ± 44.4	236.7 ± 44.8	171.9 ± 39.4	220.7 ± 72.7	0.0597	0.0097	0.6058
Linoleic (18:2 n-6)	398.3± 63.9	370.8 ± 68.2	221.3 ± 55.4	308.3 ± 117.9	0.0565	0.0003	0.3100
α-Linolenic (18:3 n-3)	32.0 ± 5.0	29.0 ± 5.4	16.6 ± 3.2	22.3 ± 7.9	0.0763	< 0.0001	0.5724
Arachidonic (20:4 n-6)	29.6 ± 6.9	28.7 ± 9.3	21.4 ± 7.9	23.0 ± 11.3	0.2537	0.0062	0.9254
Docosahexaenoic (22:6 n-3)	62.2 ± 17.9	47.5 ± 15.2	28.0 ± 15.9	36.5 ± 21.3	0.0660	0.0009	0.6131

NMDA, N-methyl-D-aspartic acid, 25 mg/kg, i.p.; LTG, lamotrigine.

Values are means ± SD measured from arterial plasma collected before [1-^{14}C] AA infusion.

Table 2

Interactive effects of chronic lamotrigine and acute NMDA on regional arachidonic acid incorporation coefficients in rat brain

Brain region	Vehicle		LTG		LTG × NMDA Interaction (p value)
	Saline (n = 7)	NMDA (n = 9)	Saline (n = 8)	NMDA (n = 8)	
	k*, (ml/s/g) × 10^{-4}				
Prefrontal cortex layer I	7.83 ± 1.50	11.53 ± 3.65	9.77 ± 2.42	7.59 ± 2.57	0.005
Prefrontal cortex layer IV	10.16 ± 1.66	14.41 ± 3.89	11.37 ± 3.00	9.96 ± 3.90	0.023
Primary olfactory cortex	9.35 ± 1.49	12.97 ± 4.13	11.25 ± 3.12	9.73 ± 3.85	0.041
Pyriform cortex	6.44 ± 2.01	10.01 ± 3.09 *	6.88 ± 1.03	5.62 ± 1.43	0.003
Anterior cingulate cortex	12.54 ± 1.95	18.59 ± 5.49	15.25 ± 3.63	12.19 ± 5.00	0.006
Motor cortex					
Layer I	7.89 ± 1.80	11.82 ± 3.17 *	10.19 ± 2.27	7.56 ± 2.67	0.001
Layer II –III	8.91 ± 1.73	13.49 ± 4.03 *	11.7 ± 2.87	8.79 ± 3.15	0.002
Layer IV	10.40 ± 1.81	15.91 ± 5.09 *	13.23 ± 3.22	10.77 ± 3.29	0.005
Layer V	9.96 ± 1.76	15.01 ± 4.80 *	12.45 ± 3.04	9.65 ± 3.74	0.005
Layer VI	9.29 ± 1.95	13.83 ± 4.07 *	11.50 ± 2.38	8.89 ± 3.25	0.003
Somatosensory cortex					
Layer I	8.70 ± 1.59	12.70 ± 3.29 *	10.77 ± 2.64	8.68 ± 3.57	0.007
Layer II – III	9.75 ± 2.25	14.83 ± 3.63 *	12.33 ± 3.05	9.68 ± 3.61	0.002
Layer IV	11.21 ± 2.09	17.82 ± 4.79 *	14.24 ± 3.75	11.39 ± 2.47	0.002
Layer V	10.08 ± 2.00	14.98 ± 4.35 *	12.83 ± 3.27	9.71 ± 3.72	0.003
Layer VI	9.74 ± 1.95	14.36 ± 4.06 *	11.72 ± 2.38	9.18 ± 3.27	0.003
Preoptic area (LPO/MPO)	6.70 ± 1.19	10.56 ± 3.62 *	8.02±1.53	7.67 ± 3.44	0.040
Globus pallidus	7.56 ± 1.45	10.59 ± 2.96	8.97 ± 1.98	7.18 ± 3.17	0.012
Diagonal band Dorsal	9.32 ± 1.73	13.77 ± 3.99 *	11.28 ± 3.40	8.32 ± 3.92	0.008
Ventral	9.47 ± 2.12	14.06 ± 3.68 *	11.35 ± 3.05	9.06 ± 4.32	0.009
Amygdala basolateral/medial	7.54 ± 1.19	11.48 ± 3.52 *	8.68 ± 2.56	6.98 ± 2.02	0.004
Hippocampus					

Brain region	Vehicle		LTG		LTG × NMDA Interaction (p value)
	Saline (n = 7)	NMDA (n = 9)	Saline (n = 8)	NMDA (n = 8)	
CA1	6.72 ± 1.23	9.75 ± 2.84**	7.95 ± 2.73	6.78 ± 2.34	0.021
CA2	7.57 ± 1.37	10.61 ± 2.82**	8.44 ± 2.72	6.75 ± 2.05	0.009
CA3	7.54 ± 1.19	10.44 ± 2.37*	8.44 ± 2.79	7.20 ± 2.13	0.017
Dentate gyrus	8.01 ± 1.54	11.14 ± 2.76*	9.23 ± 2.73	7.65 ± 2.09	0.009
Nucleus Accumbens	10.09 ± 0.72	14.30 ± 3.92*	10.03 ± 2.07	8.39 ± 1.99	0.003
Caudate putamen					
Dorsal	9.69 ± 1.63	13.65 ± 3.34*	10.97 ± 2.76	8.29 ± 3.63	0.004
Ventral	10.11 ± 1.84	14.04 ± 3.31*	11.00 ± 2.37	8.39 ± 3.89	0.005
Lateral	10.03±1.99	14.66 ± 3.49*	11.08 ± 2.69	8.37 ± 3.97	0.003
Medial	9.69 ± 2.05	13.85 ± 3.05*	11.11 ± 2.44	8.32 ± 4.22	0.004
Septal nu lateral	7.56 ± 1.64	10.05± 3.18	8.82 ± 2.07	6.00 ± 2.15	0.004
Septal nu medial	9.91 ± 2.40	13.17 ± 4.53	11.42 ± 3.06	8.12 ± 3.80	0.016
Habenular nu lateral	14.97 ± 2.57	21.09 ± 4.30*	17.17 ± 5.53	15.06 ± 2.45	0.007
Habenular nu medial	12.81 ± 1.88	16.75 ± 4.30	14.86 ± 4.61	12.49 ± 2.32	0.019
Lateral geniculate nu dorsal	11.98 ± 2.10	16.56 ± 4.21	14.13 ± 4.11	11.86 ± 2.48	0.009
Thalamus					
Ventroposterior lateral nu	10.30 ± 1.89	15.08 ± 3.71*	12.69 ± 3.47	11.11 ± 2.86	0.008
Ventroposterior medial nu	10.21 ± 1.74	15.30 ± 4.66*	12.41 ± 3.12	10.64 ± 2.72	0.007
Anteroventral nu	14.85 ± 2.30	21.65 ± 6.25*	16.54 ± 3.99	12.74 ± 3.85	0.001
Reticular nu	11.16 ± 1.89	14.38 ± 2.36*	12.62 ± 2.02	10.00 ± 2.66	0.001
Paraventricular nu	9.80 ± 1.11	12.30 ± 4.13	11.69 ± 2.12	8.88 ± 3.22	0.018
Parafascicular nu	10.56 ± 2.12	16.35 ± 4.10**	12.96 ± 3.65	11.27 ± 2.90	0.003
Subthalamic nu	11.09 ± 1.35	14.75 ± 3.38	13.58 ± 4.58	11.24 ± 2.01	0.012
Hypothalamus					
Supraoptic nu	8.09 ± 1.61	11.48 ± 3.09*	9.97 ± 2.48	7.50 ± 2.87	0.004
Lateral	8.09 ± 097	10.08 ± 1.33	9.53 ± 1.84	7.60 ± 2.86	0.007
Anterior	8.53 ± 1.23	12.02 ± 3.49	10.36 ± 1.99	8.89 ± 4.45	0.034

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Brain region	Vehicle		LTG		LTG × NMDA Interaction (p value)
	Saline (n = 7)	NMDA (n = 9)	Saline (n = 8)	NMDA (n = 8)	
Periventricular	8.23 ± 1.08	12.30 ± 3.12 **	9.95 ± 1.78	7.73 ± 3.06	0.001
Arcuate	7.86 ± 1.74	11.80 ± 3.13 *	9.10 ± 1.81	7.53 ± 2.85	0.004
Ventromedial	7.94 ± 1.74	11.99 ± 3.61 *	9.26 ± 2.05	7.51 ± 2.51	0.005
Posterior	8.92 ± 1.51	12.06 ± 2.58 *	10.91 ± 1.68	8.90 ± 2.91	0.003
Zona incerta	10.53 ± 1.57	15.53 ± 3.69 **	12.42 ± 3.85	11.35 ± 1.82	0.008
Cerebellar white matter	5.53 ± 1.09	7.60 ± 1.23	6.81 ± 2.08	5.88 ± 1.85	0.016
Non-blood-brain barrier regions					
Subfornical organ	7.65 ± 1.87	12.07 ± 4.39 *	9.24 ± 3.64	6.68 ± 2.74	0.007
Median eminence	7.89 ± 1.75	10.89 ± 2.32	9.17 ± 2.78	7.62 ± 2.81	0.016

Abbreviations: nu, nucleus; lat, lateral; med, medial; SLM, stratum lacunosum-moleculare; LTG, lamotrigine.

NMDA administration: 25 mg/kg i.p. for 10 min. Mean ± S.D.

* $p < 0.05$;

** $p < 0.01$; vehicle + NMDA *vs.* vehicle + saline (one-way ANOVA Bonferroni *post-hoc* tests).

Table 3

Effect of NMDA on brain PGE_2 and TXB_2 concentrations in chronic vehicle- and LTG-treated rats

	Chronic Vehicle		Chronic LTG		LTG × NMDA Interaction P-value
	Saline (n = 8)	NMDA (n = 8)	Saline (n = 6)	NMDA (n = 6)	
PGE_2 (ng/g brain)	5.2 ± 1.3	11.5 ± 2.8***	2.5 ± 0.6**	1.8 ± 0.5	< 0.0001
TXB_2 (pg/g brain)	27.3 ± 12.4	65.0 ± 29.0***	19.2 ± 7.1	16.0 ± 9.4	0.0067

Each value is mean ± SD. Bonferroni's multiple comparison tests were performed.

** P < 0.01;

*** P<0.001; vehicle plus NMDA versus vehicle plus saline, LTG plus saline versus vehicle plus saline, and LTG plus NMDA versus LTG plus saline.

NMDA, N-methyl-D-aspartic acid; LTG, lamotrigine.